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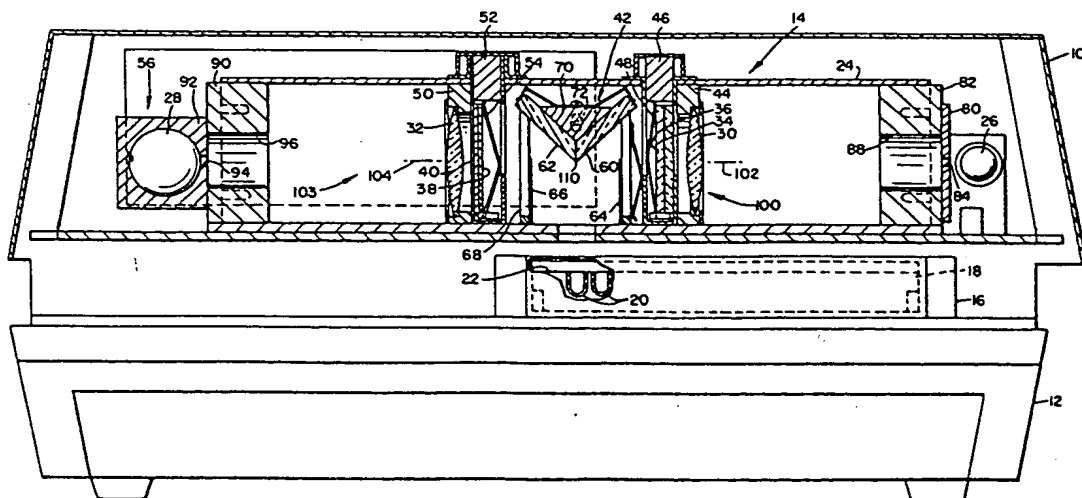
DOC

(54) Fluorometer.

(57) A sensitive frontal approach fluorometer, suitable for measuring the fluorescence of samples in open top microtest wells (20), has an optical system (14) for (a) directing an exciting light downwardly into the well's open top fluorescently to excite the sample and (b) detecting the sample's emitted light which passes upwardly through the well's open top.

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Fig. 3



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1 in U.S. Patent No. 4,154,795 which issued to A.C. Thorne
on May 15, 1979.

The use of microtest plates and microtest strips
of wells in fluorescent and other types of assays offers
5 several important advantages. First, they permit the mass
preparation of a large number of test sample solutions at
the same time. Second, they are more convenient to handle
as compared with individual test tubes. Third, they can
easily and inexpensively be washed. Fourth, they are
10 inexpensive and disposable. Fifth, they are customarily
formed from plastic materials which are not fragile like
glass. Sixth, they can be made from a material having an
ability to attract certain molecules such as protein
molecules so that they can serve as a solid phase in an
15 immunoassay.

Some fluorometers are not sufficiently sensitive
to measure the fluorescence of the small, microliter quantities
of the relatively low fluorescent samples which are prepared
with the microtest plate and strip equipment described above.
20 Other fluorometers, while having sufficient sensitivity,
are usually unsuitable for measuring the fluorescence of
substances in microtest wells because they are designed to
direct the exciting light and/or the sample's emitted light
through a wall of the sample-holding vessel. As a result,
25 the microtest plates and strips, which are customarily molded
from plastic materials having a substantial level of native
fluorescence, are excessively excited to produce spurious
light emissions which interfere with and impair accurate
measurements of the intensity of the light emitted by the
30 fluorescently excited test sample itself.

FLUOROMETER

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1 This invention relates to fluorometers and is particularly concerned with a novel optical system for a fluorometer of the frontal approach type. The fluorometer of this invention is particularly suitable for measuring
5 the fluorescence of substances in microtest wells (or microtiter wells, as they are also called) and other similar vessels.

For purposes of this specification, a fluorometer of the frontal approach type is one in which the exciting light or radiation enters through the open top of a sample-
10 holding vessel and in which the detected emitted light (resulting from fluorescent excitation of the material) exits through the vessel's open top.

The term "light" as used in this specification refers to both non-visible light (e.g., ultraviolet light)
15 and visible light, that is, light visible to the naked eye.

Prior to this invention various fluorometers have been used in a wide variety of applications for measuring the fluorescence of fluorescently excitable materials. For example, fluorometers are used in conjunction with fluorescent
20 assays to detect and measure the quantities of immunological and non-immunological substances.

In carrying out fluorescent assays, microtest plates (or microtitration plates, as they are also called) and strips
of microtest wells are often used. Microtest plates are formed
25 with a multiplicity of wells which are joined together in a molded one-piece structure for containing microliter quantities of fluid samples in liquid or solid form. Examples of a microtest plate and microtest wells in strip form are described

1 With the foregoing in mind, the general aim and purpose of this invention is to provide a novel fluorometer which meets both of the foregoing requirements, namely high sensitivity and suitability for measuring the fluorescence of
5 microliter quantities of test samples in microtest plate wells and similar vessels.

 A more specific object of this invention is to provide a frontal approach fluorometer with a novel optical system which utilizes a special focusing technique to provide for a
10 highly efficient transfer of radiation energy from an excitation light source to the sample and also to provide for a highly efficient transfer of emitted light from the fluorescently excited sample to a detector.

 In accordance with this invention, the optical
15 system comprises a pair of bi-convex lenses or double-convex lenses, as they are also called, one for transmitting the exciting light to the test sample, the other for transmitting the light emitted by the fluorescently excited material to a photodetector.

20 On the exciting light side of the optical system, the distance of the light path followed by the exciting light between the exciting light source and the sample in the microtest well or other sample-holding vessel is set to equal a multiple (e.g. four times) of the focal length of the lens
25 used for transmitting the exciting light, and the lens for the exciting light is located at the midpoint of the exciting light's path. The light path distance between the exciting light source and the lens for the

1 exciting light is therefore equal to twice the lens' focal
length. Likewise, the light path distance between the
exciting light's lens and the sample in the well is also
equal to twice the lens' focal length. The image of the
5 light source will therefore be focused sharply on the sample
in the well to maximise the fluorescent excitation of the
sample for a given intensity of the exciting light. A corres-
ponding focusing technique is applied to the lens for the
sample's emitted light.

10 Thus, on the emitted light side of the optical
system, the total length of the light path between the sample
in the well and the fluorometer's photodetector is set
to equal a multiple (e.g. four times) of the focal length
of the lens used for transmitting the sample's emitted
15 light, and the lens for the emitted light is located at
the midpoint of the light path followed by the emitted
light. The light path distance between the lens for the
emitted light and the sample in the well is therefore equal
to twice the lens' focal length, while the light path distance
20 between the lens for emitted light and the photodetector
is also equal to twice the lens' focal length so that the
full image of the sample is sharply focused on the photo-
detector to maximise the intensity of the light detected
by the photodetector.

25 Because of the foregoing focusing conditions, the
sensitivity of the optical system is significantly enhanced
as compared with systems using unfocused light.

 According to another feature of this invention
the total length of the light path for the exciting light and
30 the total length of the light path for the emitted light are _____

1 selectively and independently adjustable to compensate for
imperfections in the lenses which cause the lenses' focal
lengths to deviate from a design or ideal value. The length of
the light path for the exciting light may be adjusted by
5 adjusting the position of the object, namely the light source
or an optical stop lying in front of the light source. On the
emitted light side of the optical system, the light path for
the emitted light is adjusted by adjusting the position of
the photodetector.

10 In addition to the foregoing, the optical system of
this invention includes a novel mirror and mask arrangement
which lies between the two lenses. This mirror and mask
arrangement performs a number of important functions. First,
it downwardly reflects the exciting light to cause it to enter
15 the sample-holding well through the open top thereof. Second,
it works in conjunction with the lens for the exciting light
to direct the exciting light beam through the well's open top
without striking the well's side wall or the surface regions
around the open top of the well. Third, it reflects the sample's
20 emitted light, which passes upwardly through the well's open top,
to cause it to pass through the lens for the emitted light.
Fourth, it keeps the sample's emitted light from entering the
exciting light channel lying between the mirror arrangement and
the exciting light source, and it also keeps the exciting light
25 from entering the emitted light channel lying between the mirror
arrangement and the photodetector. Finally, it reduces the
amount of scattered radiation in the emitted light channel to
reduce the noise signal level in the fluorometer's photodetector.

In order that the invention may be more readily

1 understood, reference will now be made to the accompanying drawings, in which:-

Figure 1 is a front elevation of a fluorometer incorporating the principles of this invention and showing the
5 outer cabinet partially broken away to illustrate the optical system's inner housing;

Figure 2 is a top plan view of the fluorometer shown in Figure 1, with the top cabinet wall broken away to illustrate the optical system's housing;

10 Figure 3 is a section taken substantially along lines 3-3 of Figure 2; and

Figure 4 is an elevation of the illustrated optical system in partially schematic form.

Referring to the drawings and particularly to
15 Figures 1 and 2, the fluorometer incorporating the principles of this invention comprises an outer cabinet 10, a support base 12, an optical system 14, and a movable carriage 16 for supporting a microtest plate 18. Cabinet 10 is preferably light tight. Base 12 supports carriage 16.

20 Microtest plate 18 contains a multiplicity of open top wells 20 for receiving and holding test samples in liquid form. Plate 18 may be of the type shown in the previously mentioned Patent No. 4,154,795 or it may be of the type shown in U.S. Patent No. 3,356,462 which issued to N. M. Cooke et al
25 on December 5, 1967. The disclosures of these patents are incorporated into this specification by reference.

Wells 20 are uniformly spaced apart in twelve parallel spaced apart rows of wells with eight wells in each

1 row to provide the standard total of 96 wells. Each of the wells 20 is formed with a cylindrical side wall and a suitable bottom wall. Wells 20 depend from a top wall 22 of the plate.

The carriage 16 together with plate 18 and optical 5 system 14 are all mounted in cabinet 10. Carriage 16 lies below the optical system 14 as shown.

Referring now to Figures (3) and (4), optical system 14 comprises an elongated housing 24 of rectangular cross-section, a suitable source of exciting light or radiation such 10 as an ultraviolet lamp 26, a suitable photodetector such as a photomultiplier 28, a pair of bi-convex lenses 30 and 32, a first pair of filters 34 and 36, a second pair of filters 38 and 40, and a mirror and optical mask assembly 42.

As shown in Figure (3), lens 30 is mounted in a 15 suitable holder 44. Filters 34 and 36 are mounted side by side in another suitable holder 46 and are releasably retained in place by a leaf spring 48.

Lens 32 is also mounted in a suitable holder 50. Filters 38 and 40 are mounted side by side in another suitable 20 holder 52 and are retained in place by a leaf spring 54.

Holders 44, 46, 50 and 52 are all mounted in housing 24 as shown. Holder 46 and filters 34 and 36 are removable as a unit through an opening in the top of housing 24. Similarly, holder 52 and filters 38 and 40 are also 25 removable as a unit through another opening in the top wall of housing 24.

Still referring to Figure (3), lamp 26 is mounted exteriorly of housing 24 at one end thereof, and photomultiplier 28 is mounted in a holder 56 at the opposite end of housing 24.

1 The mirror and mask assembly 42 is mounted in housing 24 centrally between the housing's opposite ends. As shown, mirror and mask assembly 42 comprises a pair of light-reflecting mirrors 60 and 62 and a pair of optical
5 masks 64 and 66. Masks 64 and 66 are rigidly mounted on a support frame 68 which in turn is mounted in housing 24. Mirrors 60 and 62 are mounted on a support member 70 which in turn is mounted on frame 68 by means of a screw and slot connection 72, which permits selective vertical adjustment of the
10 assembly of mirrors 60 and 62 and support member 70.

The assembly of mirrors 60 and 62 defines a V-shaped configuration in which one of the mirrors forms one leg of the V-shaped configuration and the other mirror forms the other leg of the V-shaped configuration. Mirrors 60 and 62 abut against
15 each other at the apex of the V-shaped configuration. The angle included between mirrors 60 and 62 is preferably 90° . Mirrors 60 and 62 are symmetrical about a vertical plane passing through the interface between the apex-defining, abutting edges of the mirrors. The apex defined by the abutting ends of
20 the mirrors 60 and 62 is indicated at 110 and lies vertically above an aperture 76 which is formed through the bottom wall of housing 24 above plate 18 in carriage 16. The wall region defining aperture 76 constitutes an optical stop.

Still referring to Figure 3, a further optical stop
25 80 is mounted on the outer end of a support block 82 which is slidably received in the end of housing 24 adjacent to lamp 26. The optical stop 80 is formed with a central opening 84 lying along an axis which normally intersects the

1 longitudinal axis of lamp 26. Aperture 84 is axially aligned with and opens into an enlarged aperture 88 which is formed through support block 82. Optical stop 80 lies on the outer side of housing 24 between lamp 26 and support block 82.

5 Aperture 84 lies closely adjacent to lamp 26 as shown. The diameter of the support block's aperture 88 is substantially larger than that of aperture 82 to allow the rays of the exciting light passing through aperture 84 from lamp 26 to diverge in the manner shown in Figure 4.

10 Still referring to Figure 3, the photomultiplier holder 56 is mounted on the outer end of another support block 90 which is slidably received in the end of housing 24 opposite from support block 82. The wall region of holder 56 abutting support block 90 defines another optical stop 92 having a
15 central light-transmitting aperture 94. The inner end of aperture 94 axially aligns with an enlarged aperture 96 which is formed through support block 90. Aperture 96 is sufficiently large in diameter to allow converging light rays from lens
32 to enter aperture 94 without being blocked. Aperture 94
20 establishes the light path for transmitting light from lens 32 to photomultiplier 28.

Holder 56 is sufficiently large to cover the aperture 96 in support block 90. Similarly, optical stop 80 is sufficiently large to cover the aperture 88 in block 82 except
25 for the opening provided by aperture 84. Housing 24 is preferably light tight except for apertures 84 and 94.

As shown in Figures 3 and 4, lens 30 and filters 34 and 36 are arranged between optical stop 80 and mirror 60 to form an exciting light channel 100 for system 14. The principal

1 axis or centerline of lens 30 is indicated at 102 in Figures 3
and 4 and axially aligns with apertures 84 and 88. Lens 30
is positioned between optical stop 80 and filter 34. Lens 30
lies closely adjacent to filter 34 so that the spacing between
5 lens 30 and filter 34 is considerably smaller than the spacing
between lens 30 and optical stop 80. Filter 36 is positioned
between filter 34 and mirror 60 and is spaced from mirror 60
as shown. Filter 36 may abut against filter 34 as shown.

Still referring to Figures 3 and 4, lens 32 and
10 filters 38 and 40 are arranged between mirror 62 and optical
stop 92 to form an emitted light channel 103 for system 14.
The principal axis or centerline of lens 32 is indicated at
104 and axially aligns with the axes of apertures 94 and 96.
In the illustrated embodiment, the principal axis 104 of lens
15 32 also axially aligns with the principal axis 102 of lens 30.

Lens 32 is positioned between filter 40 and aperture
94 and lies closely adjacent to filter 40 so that the distance
between lens 32 and filter 40 is considerably smaller than
the distance between lens 32 and aperture 94.

20 As shown in Figure 4, light emitted by lamp 26
passes through the optical stop's aperture 84. From there, the
rays of the exciting light diverge to lens 30. These light rays
are refracted by lens 30 so that the light rays passing beyond
the lens converge towards mirror 60 and pass through filters
25 34 and 36. In this embodiment, filters 34 and 36 pass just
ultraviolet light, while rejecting all other wave lengths.

The apex 110 of mirror assembly 60, 62 lies on the
aligned principal axes 102 and 104 of lenses 30 and 32.

1 Mirrors 60 and 62 and masks 64 and 66 are symmetrically
arranged about a vertical plane passing through the mirrors'
apex 110 and containing the longitudinal axis of aperture 76.
Mask 64 is located vertically below the reflecting surface of
5 mirror 60 and has its upper edge lying just above the aligned
principal axes of the lenses so that it lies just above the
level of the apex 110. Accordingly, light passing through the
lower half of lens 30 below the principal axis 102 will be
blocked by mask 64, thus preventing the exciting light from
10 passing into the system's emitted light channel 103.

Because of the foregoing arrangement of mirror 60
and mask 64, only the light passing through the upper half of
lens 30 will strike and be reflected by mirror 60. The con-
verging column of light striking mirror 60 will be reflected
15 downwardly at a small acute angle to a vertical plane because
of the 45° angle which the reflecting surface of mirror 60
makes with the principal axis of lens 30.

The column of excited light reflected by mirror 60
passes downwardly through aperture 76 and through the open top
20 of one of the sample-holding wells 20 which is selectively
positioned to lie vertically below aperture 76 in alignment
with the longitudinal axis of aperture 76. The column of
exciting light entering well 20 strikes the test sample in the
well. As a result, the fluorescently excitable substance or
25 substances in the test sample will be fluorescently excited
to emit light which passes upwardly through the open top of
well 20 and through aperture 76 to strike the reflecting
surface of mirror 62. The reflecting surface of mirror 62

1 intersects the lenses' principal axes 102, 104 at a 45° angle.

Because of the angulation of the reflecting surface of mirror 62, the rays of the sample's emitted light striking mirror 62 will be reflected towards lens 32 and will diverge in the direction of lens 32 as shown in Figure (4). The diverging rays of light reflected from mirror 62 pass through filters 38 and 40 before arriving at lens 32.

In the illustrated embodiment, filter 38 is designed to reject light in the ultraviolet range while passing all other wave lengths above the ultraviolet range. Filter 40 is of the band pass type for passing just one preselected wave length (or a narrow wave length band) of the emitted light passed by filter 38. The light wave length passed by filter 40 is selected to measure the fluorescence of light emitted by a particular substance of interest in the sample in well 20.

Mask 66 is positioned vertically below the reflecting surface of mirror 62 and has its upper edge lying just slightly above the level of the mirror apex 110. Mask 66 is positioned between mask 64 and the pack of filters 38, 40 to block transmission of stray light at and below the aligned principal axes 102 and 104 of the lenses. Accordingly, the only light transmitted to lens 32 will lie above the aligned principal axes of the lenses. Mask 64 blocks the entry of emitted light and any stray light into the system's exciting light channel 100.

The rays of the sample's emitted light entering lens 32 will be refracted by lens 32 such that the light rays leaving lens 32 will converge virtually to a point in the

1 optical stop's aperture 94 which directs the sample's emitted light to photomultiplier 28 for measurement.

Photomultiplier 28 measures the intensity of the sample's emitted light. The measured intensity of the
5 emitted light in turn is a measure of the quantity of the fluorescently excited substance which produced the emitted light at the wave length passed by filter 40.

Preferably, lenses 30 and 32 are the same and have equal focal lengths.

10 In the illustrated embodiment, the object "seen" by lens 30 is the exciting light passing through the optical stop's aperture 84. The exciting light passing through aperture 84 represents the light source viewed from lens 30.

In accordance with this invention, the length of
15 the path followed by the exciting light from aperture 84 to a desired image point or location in the sample-holding well 20 (as measured along the principal axis from aperture 84 to mirror 60 and from mirror 60 to well 20) is set to equal or at least substantially equal four times the design focal length
20 of lens 30. Lens 30 is positioned at the midpoint of this path. Because of this arrangement, the length of the foregoing path between aperture 84 and lens 30 will be equal to twice the design focal length of lens 30. Likewise, the length of the foregoing path between lens 30 and desired image location in
25 well 20 is also equal to twice the design focal length of lens 30. This image location in well 20 is selected so that it lies at or at least closely at the surface of the sample in well 20.

1 Accordingly, where the object (the light source) lies at spot f_1 in aperture 84, the sharply focused image will appear at spot f_2 centrally in well 20 as shown in Figure 4.

Because of the optical system thus far described,
5 the image of the exciting light will be sharply focused centrally in well 20 on the sample in well 20 without causing the downwardly reflected exciting light column to strike the side wall of the well or the surface region of plate 18 around the open top of the targeted well. Fluorescent excitation of the
10 sample will therefore be maximized for a given intensity of the exciting light to enhance or strengthen the light emitted by the fluorescently excited sample. In addition, fluorescent excitation of the microtest plate will be reduced by directing the downwardly reflected exciting column into well 20 without
15 striking the well's side wall or the top wall of plate 18.

The length of the path travelled by the exciting light from the optical stop's aperture 84 to the image location in well 20 is selectively adjustable to compensate for imperfections in lens 30. Such imperfections cause small deviates
20 in the lens' focal length from the design or ideal length.

In the illustrated embodiment the foregoing adjustment is accomplished by selectively adjusting the position of optical stop 80 along the principal axis of lens 30. Any suitable means may be employed for adjusting the optical stop
25 80.

For example, support block 82 may be releasably fixed in place by screws 120 extending through horizontally elongated slots 122 in housing 24 and threaded into tapped

1 bores in block 82 as shown in Figure 2. If the image of the
exciting light is not precisely focused on the desired location
in well 20 after system 14 is assembled, screws 120 may be
loosened to allow the assembly of block 82 and optical stop 80
5 to be shifted to a new position along the lens' principal axis
where the image of the exciting light focuses more sharply at
the desired location in well 20.

The same focusing techniques used for the exciting
channel 100 are applied to the emitted light channel 103. In
10 particular, the length of the path followed by the emitted
light from the sample in well 20 to a desired image location
at the optical stop's aperture 94 (as measured along the
principal axis from aperture 94 to mirror 62 and from mirror
62 to well 20) is set to equal or at least substantially equal
15 four times the design focal length of lens 32. Lens 32 is
positioned at the midpoint of this path. Because of this
arrangement, the length of the path between aperture 94 and
lens 32 will be equal to twice the design focal length of
lens 32. Likewise, the length of the path between lens 32
20 and the sample in well 20 is also equal to twice the design
focal length of lens 32. Accordingly, where the object (the
sample) lies at spot f_3 in well 20 the sharply focused image
of the sample will appear at spot f_4 in the aperture 94, all
as shown in Figure 4.

25 Because of the foregoing arrangement, substantially
the full image of the fluorescently excited sample in well 20
will be sharply focused in aperture 94 and thus on photo-
multiplier 28 to maximize the intensity of the emitted light
detected by the photomultiplier.

1 Similar to support block 82, support block 90 also
is mounted for selective adjustment along the principal axis
of lens 32 by screws 124 extending through horizontally elon-
gated slots 126 in housing 24 and threaded into tapped bores
5 in block 90. If the emitted light lens 32 is not precisely
focused on the desired location in well 20, screws 124 may be
loosened to allow the assembly of block 90, photomultiplier
holder 92 and photomultiplier 28 to be shifted as a unit along
the principal axis of lens 32 to a new position where the image
10 of the emitted light focuses more sharply on photomultiplier
tube 28. In checking for the focus for lens 32, the photo-
multiplier tube 28 may be replaced by a lamp, thus becoming
a light source type of object for lens 32 to provide for the
focusing of the lamp's image in well 20.

15 Although the focus adjustments described for
channels 100 and 103 are advantageous for obtaining optimum
focusing they are optional in the sense that satisfactory
focusing can be achieved in the initial assembly of the compo-
nent parts of the fluorometer.

20 In summary, it will be appreciated that the down-
wardly reflected exciting light beam is directed and confined
to strike the sample in well 20 without striking the well's
side wall or the top surface of plate 18. It also will be
appreciated that photomultiplier 28 detects just those rays
25 of the sample's emitted light passing upwardly through the
open top of well 20.

The fluorometer of this invention is therefore
particularly suitable for measuring the fluorescence of sub-
stances in microtest wells making it unnecessary to transfer

1 samples prepared in microtest plates or strips to special
cuvettes or tubes for holding the samples during the fluoro-
metric measurements.

Where the sample-holding microtest plate or strip
5 exhibits a substantial level of fluorescence when exposed to
the exciting light in the fluorometer, it will be appreciated
that a reference reading may be taken of the plate's native
fluorescence to adjust the fluorometric measurements of the
samples. Alternatively or additionally, the operator may use
10 non-fluorescent or low-fluorescent microtest plates or strips
of the type described in our co-pending EPC application
(corresponding to U.S. patent application No. 433,826)
filed on even date herewith for Non-Fluorescent Vessels For
Holding Test Samples in Fluorescent Assays.

15 In view of the foregoing, it will be appreciated
that the samples may be prepared in microtest plate 18 and
that the plate may then be placed in the fluorometer of this
invention for individually measuring the fluorescence of the
samples in wells 20. Any suitable, conventional mechanism
20 may be utilized for shifting carriage 16 in an X-Y plane to
individually and sequentially target the wells 20 for fluoro-
metric measurement to obtain separate fluorometric measurements
of the samples in plate 18. Alternatively, it is evident that
carriage 16 could be shifted manually to individually target
25 the samples in plate 18.

1. From the foregoing description it will be appreciated that lens 30 produces a spot image of the exciting light (as seen at aperture 84) in well 20 at spot f_2 . The diameter of aperture 84 is such that the diameter of spot
5 image produced in well 20 is nearly equal to or approaches the diameter of well 20 to excite a maximum area of the sample without causing the downwardly reflected, image-producing exciting light beam to strike the well's side wall of plate's top wall 22 before striking the sample in well 20. For a non-
10 magnifying lens and a $\frac{1}{4}$ inch diameter well, the diameter of aperture may be lightly less than $\frac{1}{4}$ inch.

The diameter of aperture 94 is slightly smaller than the diameter of well 20 or larger if lens 32 is of the type which magnifies the object.

15 Finally, it will be appreciated that the downwardly reflected, converging beam or column of exciting light enters the open circular top of well 20 at a small acute angle with well's vertically positioned longitudinal axis.

CLAIMS

- 1 1. A fluorometer for measuring the fluorescence of a material
in an open top microtest well or other sample-holding vessel
(20) characterised by support means (16) for supporting the
structure defining the vessel, a source (26) of exciting
5 light, first means (30,60) for passing a beam of said
exciting light downwardly through the open top of the
vessel to strike and fluorescently excite the material
therein without first striking the side wall of the vessel
or the surface regions around its open top when said vessel
10 is positioned at a preselected location on said support
means, a photodetector (28), and second means (32,62)
for directing the light which is emitted by the fluorescently
excited material and which passes upwardly through the
open top of the vessel to the photodetector for detection
15 thereby, said first means including a first lens (30)
and said second means including a second lens (32).
2. A fluorometer according to claim 1, characterised
in that the first and second lenses (30,32) are double
convex lenses.
- 20 3. A fluorometer according to claim 1 or 2, characterised
in that the length of the light path from the source (26)
of exciting light to the sample in the vessel (20) is
arranged to be substantially equal to a multiple of the
focal length of the first lens (30), said first lens being
25 located substantially at the midpoint of said exciting
light path.
4. A fluorometer according to claim 1, 2 or 3, characterised
in that the length of the light path between the sample
in the vessel (20) and the photodetector (28) is arranged
30 to be substantially equal to a multiple of the focal length
of the second lens (32) said second lens being located
substantially at the midpoint of the light path of the
emitted light.

- 1 5. A fluorometer according to any preceding claim, characterised in that the lengths of the light paths for the exciting and emitted light are selectively and independently adjustable to compensate for imperfections in the lenses.
- 5 6. A fluorometer according to claim 5, characterised in that the light source (26) and/or photodetector (28) are adjustably mounted in order to adjust the lengths of said light paths.
- 10 7. A fluorometer according to any preceding claim, characterised by a mirror and mask arrangement (60-66) disposed between the two lenses (30,32) for reflecting the exciting light into the vessel through the open top thereof without striking the vessel's side walls or the surface regions around its open top, and for reflecting the emitted light
- 15 through the second lens onto the photodetector (28), said arrangement being adapted to prevent the emitted light from entering the exciting light path lying between the mirror arrangement and the exciting light source (26) and also to prevent the exciting light from entering the
- 20 emitted light path lying between the mirror arrangement and the photodetector.

1 / 3

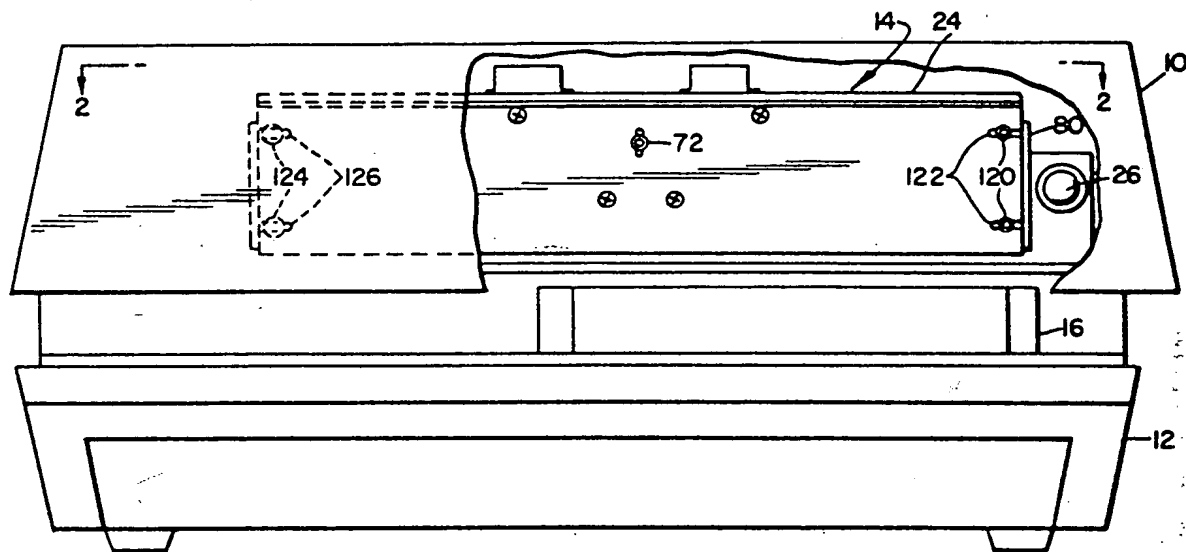


Fig. 1

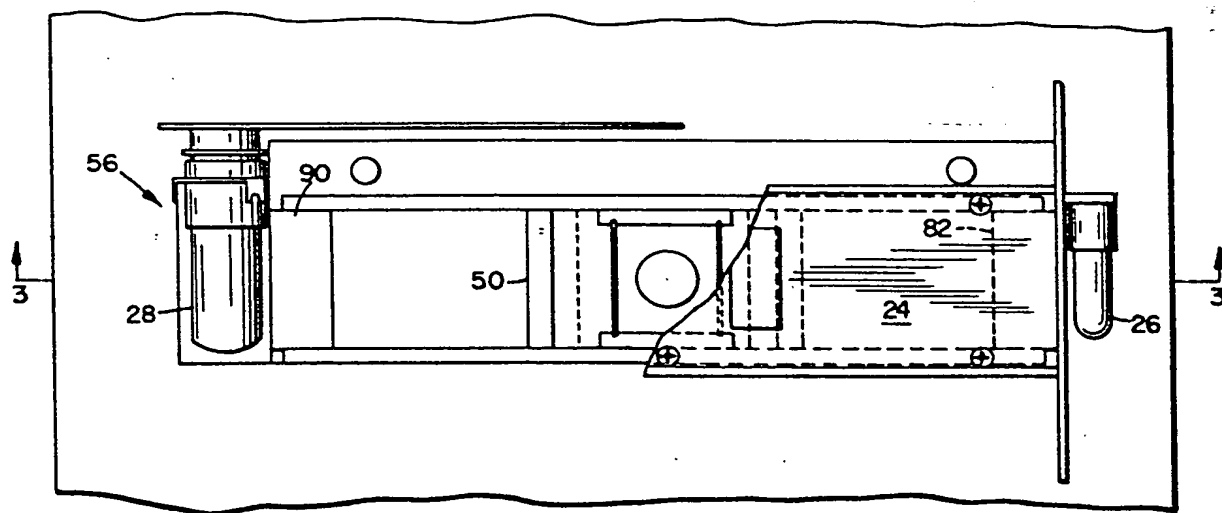
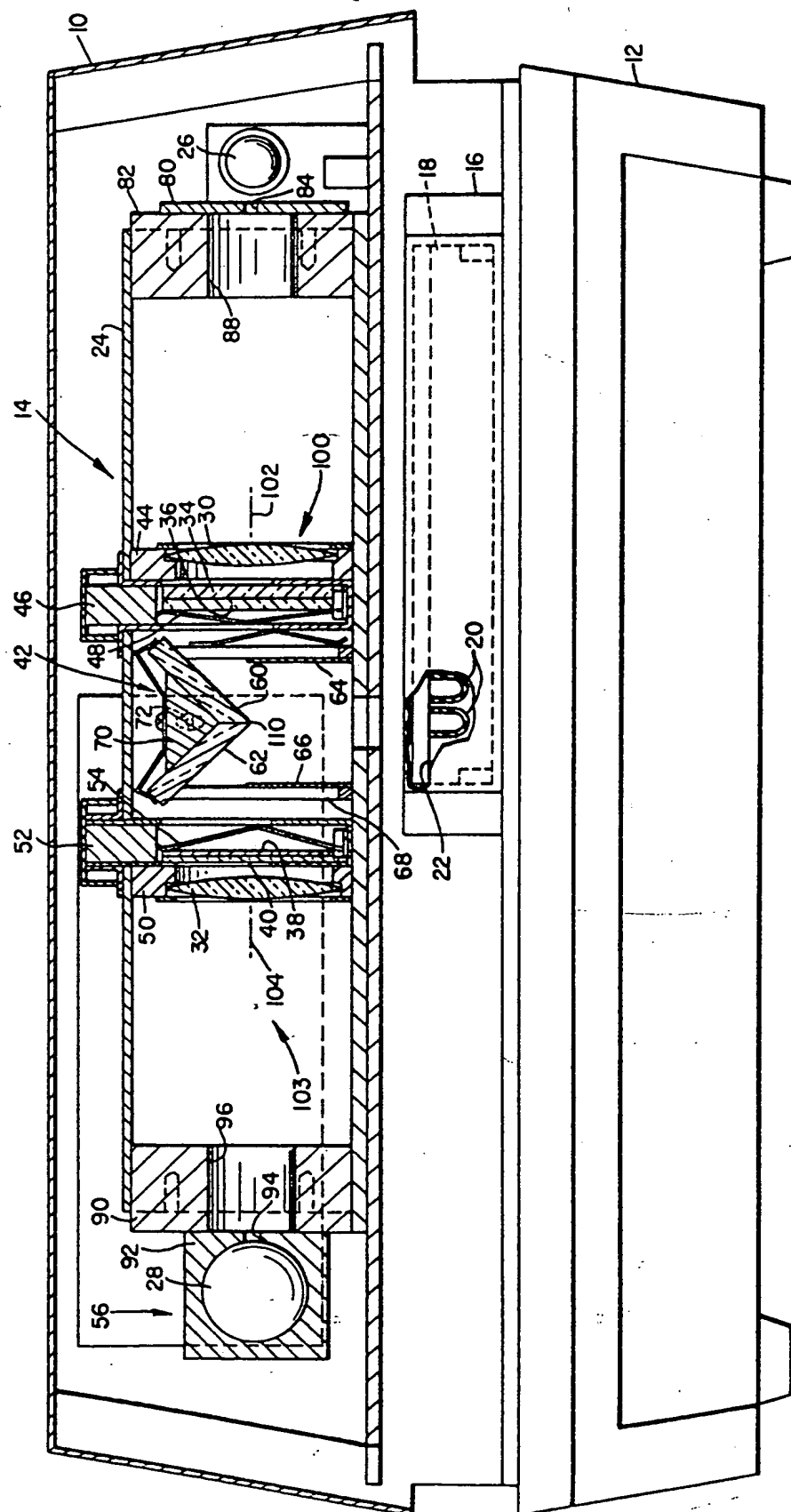


Fig. 2

Fig. 3



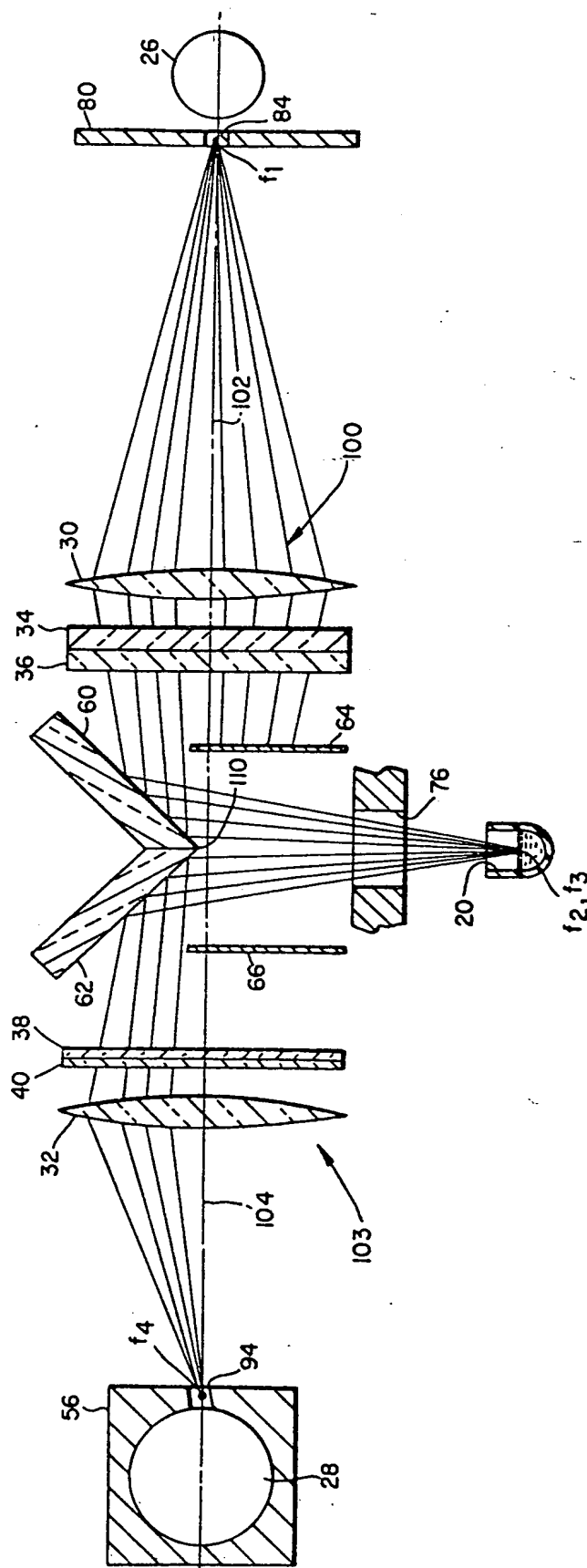


Fig. 4



European Patent
Office

EUROPEAN SEARCH REPORT

0108524

Application number

EP 83 30 6179

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
Y	DD-A- 149 574 (ZI FÜR KREBSFORSCHUNG DER ADW DER DDR) * Claims 1, 2, 4; abstract; pages 2-3; figure *	1,7	G 01 N 21/64
Y	FR-A-2 466 015 (GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG MBH) * Claims 1, 4; page 6, lines 7-26; figure 2 *	1,7	
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The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 10-01-1984	Examiner HOFMANN D G
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